# TURNING WATER INTO OIL - PHYSIOLOGY AND EFFICIENCY

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## Summary

The vision for 2020 is to double the irrigation efficiency of Australia's avocado industry. Our knowledge of how the avocado tree responds to drought raises possibilities for developing new irrigation scheduling strategies for avocados. The foundation for this development will be the management of partially dry root systems that break the traditional link between depressed tree physiology and depressed yield. This should allow us to reduce water supply by 30 to 40%. Work is needed to develop these new approaches and lift yields to match the sustained leaf physiology of the tree that exists when only half of the root system is kept wet. The possibility that this can be achieved by alternating the water supply between the two sides of the tree should be investigated.

## Introduction

The Australian avocado industry is spread across a range of environments and soil types, from the Atherton Tableland, Q, (Lat 17˚S) to Mildura, Vic, to Pemberton, WA, (Lat 35˚S). Across these environments, seasonal rainfall and evaporation patterns may vary from wet summers and dry springs to hot dry summers and cool wet winters. Thus, in many environments, water supply to the trees is an important issue. In some regions, irrigation is supplementary and in other regions it is essential for the production of a good crop. In this diversity, a single recipe for irrigation scheduling is unlikely to be appropriate, but some guidelines are needed to improve irrigation efficiency. Besides the importance of irrigation on individual properties, there is a need to develop sustainable management practices and demonstrate that, as an industry, we are responsibly using our valuable water resources.

Knowledge of how the avocado tree responds to dry spells is important for managing irrigation scheduling. In an earlier review the need for irrigation practices to be based on a sound knowledge of the tree, soil and environment was emphasised (Turner 1995). An opportunity to gather new information was provided by the funding, by Horticulture Australia (ex HRDC), of the Avocado Irrigation Project from January 1998 to June 2001. Here, we look at some of the findings of this project in relation to the physiology of the trees and the efficiency of irrigation. What have we learnt and where do we go from here?

Armed with our new knowledge from the avocado irrigation project, we can propose a vision for 2020 that fits the theme of this conference:

**To double irrigation efficiency in the Australian avocado industry by 2020.**

Here, we will discuss how we might move towards this objective. Our ability to achieve it will depend, in part, on our knowledge of the link between physiology and efficiency and this is the main issue of this paper.

### *Efficiency*

We need to be clear what we mean by efficiency, because our definition will determine the sorts of questions we ask and the likely answers. Efficiency is output/input. At the orchard level, the output might be yield and, for irrigation, the input might be mm of water added. Thus, the units of efficiency will be kg of fruit/mm of water. At the tree level, the output might be kg of fruit per tree and the input might be the total water (rain and irrigation) received. Then, efficiency will be expressed as kg/L. At an economic level, which is beyond the scope of this paper, output might be the dollars received for the fruit and an input might be the dollar cost of the irrigation. Then, efficiency will be $/$, and we hope that it is greater than 1.0!

Since efficiency is calculated as a ratio, we can increase it by either increasing outputs, with no change in inputs, or decreasing inputs, with no change in outputs. For irrigation, we can increase its efficiency by either increasing outputs, for example by adopting improved orchard practices, or we can increase it be reducing inputs. The latter is appealing for irrigation, especially to reduce wastage of water.

### *Physiology*

Physiology is about how living things work. An important consideration for us is how physiology is linked to tree performance under different irrigation regimes. And then, how might we manage that link to increase efficiency. In the avocado irrigation project, we worked towards understanding avocado physiology under water deficit so that we could use it to manipulate irrigation efficiency.

## **The avocado irrigation project**

### *Objective*

The project focussed on the management question: If a limited supply of water is available, how and when is it best used to maximise yield and quality?

### *Research approach*

We formulated questions that had physiological meaning, but also had answers that were of importance in irrigation scheduling. For experiments in the field, we needed to be sure that a dry period would occur during our experiments and so Western Australia (WA) was a logical choice. In WA, we are certain of long, hot dry summers and cool wet winters. The soils in WA, on the Swan Coastal Plain where many avocados are grown, are deep sands with low water holding capacity, increasing our chances of observing the effects of soil water deficit in field experiments.

The soils and environment of much of the avocado industry in Eastern Australia are quite different from those in the West. Therefore, we worked in the 'dry' spring at Glasshouse Mountains, Qld. Despite these choices, we needed to use plastic sheeting over the ground in field experiments to ensure root systems experienced dry conditions. We measured the soil water content to be sure that our 'dry' treatments really were dry.

Depending on the question, we used laboratory and glasshouse experiments to determine what happened under controlled conditions. We can maintain dry conditions in a glasshouse, even if it is raining outside (unless the roof leaks!). Thus, we could be more certain that the effect we were observing was associated with the treatment we applied, and not with an artifact. However, a concern was whether the results we obtained in our glasshouse experiments, using potted plants, could be transferred to trees growing in the field. To deal with this important issue, we used field experiments to verify the results of our more controlled, glasshouse experiments. In addition, field experiments provided the possibility of measuring yield.

In field experiments, we used small numbers of plants in each treatment and measured them intensively, rather than use many trees and take few measurements. In this way we determined in detail how the trees were performing.

## The questions

We asked these questions:

1. Is reproductive growth more sensitive to water deficit than vegetative growth?
2. What aspect of leaf physiology or growth is most sensitive to water deficit?
3. Does partial drying of the root system have a role in increasing irrigation efficiency in avocado?

## The experiments

### *Reproductive and vegetative growth*

Question 1 has two components: should water always be in good supply at flowering? and, is there an interaction between self and cross-pollination and water supply?

Experiments were conducted in glasshouses in WA and Qld using potted cv Hass avocado trees. We withheld water from the plants for a few days and measured the water status of leaf tissues and stems. We measured the growth rates of young expanding leaves and of growing peduncles on inflorescences. In Qld, we withheld water from potted trees on which flowers had been hand-pollinated either with self-pollen (self pollinated) or with non-self pollen (cross pollinated). In WA we used Hass trees growing in the field, some of which grew near other cultivars and others that were surrounded by Hass trees only. We assumed these two populations represented 'cross-pollinated' and 'self-pollinated' trees. However, there are uncertainties about this assumption. In each location, some trees received 1/3$^{rd}$ less water than the control trees, that received 120% of class A pan evaporation. At Glasshouse Mountains, Qld, we withheld water from trees in the field for almost 6 weeks and measured changes in soil water content, leaf water status and leaf, shoot and fruit growth.

### *Leaf physiology and soil water deficits*

The practical issue in Question 2 is whether a plant-based system can be used for scheduling irrigation. If we discover the process that is most sensitive to water deficit, this might be used to schedule irrigation. In all experiments in the glasshouse and field, we measured leaf gas exchange, which includes leaf net photosynthesis, stomatal conductance and transpiration. These measurements were usually taken daily, but on some occasions they were measured at hourly intervals to see how the gas exchange of the leaf changed during the day. We used the diurnal data to check that the time of measurement for our daily measurements was satisfactory.

### *Partial root system drying*

In practice, this strategy might allow us to reduce water application by 30 to 40% without a heavy penalty in yield. This would boost irrigation efficiency. Partial root system drying has its origins with plant physiologists, who, more than a decade ago, wanted to know whether water deficit operated alone, or in concert with other compounds generated by the plant when it experiences water deficit. If water acts alone then reduced water supply will educe the turgor pressure of leaves, close stomata, and reduce photosynthesis and consequently growth (see early text books on plant water relations, eg Kramer 1969). However, plants are more complex than this and often, when droughted plants are re-watered, the water status of the leaves is restored quickly, but stomata did not reopen for several days. To see what was going on, physiologists grew each half of the root system in separate containers and allowed only one side to dry. This strategy maintained the water status of the whole plant but exposed half the roots to drying soil. Using the example of apple (Gowing et al. 1990), a partially dry root system reduced growth, but the tissues were fully hydrated. Moreover, when Gowing et al. severed the roots on the dry side, growth returned to control levels. They interpreted this as a positive signal being transmitted from the roots exposed to dry soil, to the shoots. This is a cunning 'early warning' system for the plant that enables it to sense dry conditions, before the water supply gets low. The 'signal' is, in many cases, believed to be abscisic acid, but other compounds may be involved (Davies and Zhang 1991).

With grapevines, a field strategy has been developed to manage canopy growth using partial root drying. Water is withheld from half the root system for about three weeks before re-watering, at which time the other half is allowed to dry. This maintains a stream of 'root signals' that reduce excessive growth. If it rains, the effect disappears. Like Regulated Deficit Irrigation (RDI) in temperate fruit crops (Chalmers et al. 1984a, b), partial root drying can be used in grapes to manage growth. RDI works because the period of rapid growth of the fruit is separated in time from the growth of the shoots. Thus, RDI can be used to manage shoot growth, without a yield penalty. Such a situation does not exist for avocado and so we need to approach partial root system drying with other objectives.

Among some horticultural crops, we have used the split root system technique to gain evidence for root signals. We believe they exist in banana (Thomas 1995) and passionfruit (Turner et al. 1996) but not in roses (Mosher 1998) and we are still thinking about avocado!

In avocado, the appeal of partial root system drying is to improve irrigation efficiency by reducing the amount of water needed, hopefully without the complications of reduced yield. We do not wish to induce a 'root signal' in the plants to manage growth. Indeed this would be difficult because fruit and vegetative growth are not separated in time. However, if there was a shortage of water during a dry spell, then partial root system drying might be one way to maintain the water status of the trees by using less water. With this in mind, we conducted two experiments in WA, one in controlled conditions in a glasshouse and the other in the field at Carabooda.

In each case three treatments were applied: wet/wet, wet/dry and dry/dry. The wet/wet is the control and with the dry/dry we expect trees to experience a water deficit and generate root signals. The wet/dry treatment will separate the effects of water deficit from the root signals. In the literature, and in our own studies, we have used short term drying cycles lasting a week or so. In our avocado experiments, we maintained the treatments for several weeks in the case of the potted plants in the glasshouse and for several months in field grown trees. In this case, our objective was to look at the longer term effects of these treatments.

In the glasshouse experiment, we monitored soil water content, plant water status, leaf gas exchange, shoot and leaf growth for the first four weeks of drying. At seven weeks, we cut the plants up and examined the xylem vessels in the stems. We evaluated the hydraulic conductivity of the stems using direct examination under the microscope, fluorescent dyes and pressurised water flow through the stems. In the field experiment, which began in February, the hottest time of the year, we made a similar but less intensive series of measurements. In addition, yield was recorded in the following spring and notes made on the flowering of the trees.

## What did we discover?

*Reproductive and vegetative growth*

The drying of the whole root system to 50 cm depth on trees at Glasshouse Mountains took 30 days. By then, transpiration of the leaves had stopped but the young fruit (~ 30 mm long) continued to grow (Fig 1 and 2).

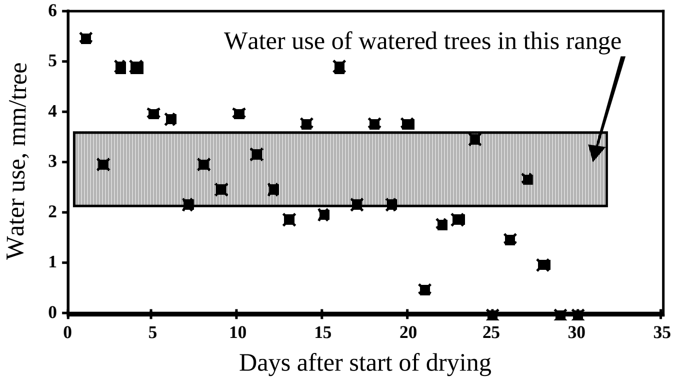

Fig 1. The water use of an unwatered avocado tree at Glasshouse Mtns, Q, in Oct-Nov 1998, fell below that of well watered trees after 20 days. Water use was measured as loss of water from the soil beneath the tree to a depth of 50 cm.

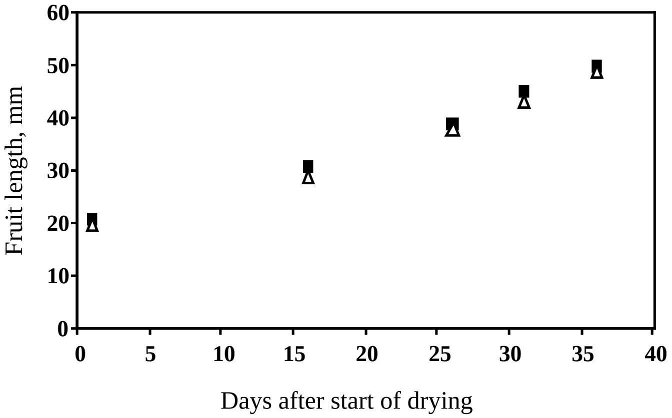

Fig 2. Withholding water from the avocado trees at Glasshouse Mtns, Q, in October/November 1998, did not reduce fruit growth. Fruit length was the same for watered (open symbols) and unwatered trees (closed symbols).

In glasshouse experiments in WA, mild stress reduced the growth of expanding leaves before it reduced the growth of expanding peduncles on the inflorescences. When potted plants with inflorescences at anthesis experienced severe drought, all flowers opened partially and aborted. In the glasshouse experiment in Qld, self-pollinated fruit fell from the plants earlier than the fruit on cross-pollinated plants that experienced the same soil water deficit. This behaviour was consistent across water treatments and encouraged us to conduct the field experiment in WA. There, we found that 'cross-pollination' increased the retention of fruit in the well-watered and stressed trees (Fig 3), indeed, 'cross pollination' compensated for the reduced water supply. Thus, our observations in the glasshouse in Qld seem to have been confirmed in the field at Carabooda, WA.

### *Leaf physiology and soil water deficits*

We expected that the expansion rate of young leaves would be the earliest process affected by water deficits (Hsiao 1973). However, in all our experiments in the glasshouse and the field, water deficits caused stomates to close before we were able to detect any other changes. In Glasshouse Mountains, Q, stomates began to close after 20 days of drying whereas fruit was still growing after 30 days of drying, compared with watered trees. In glasshouse experiments stomata closed after 4 or 5 days and in the field in WA they usually responded to drying within a few days in summer.

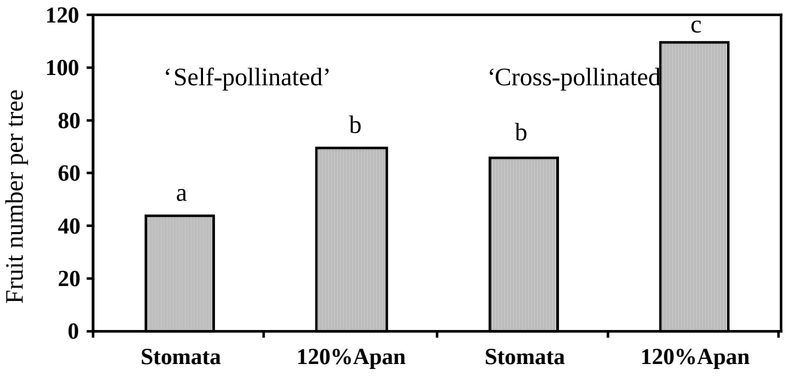

Fig. 3. Reducing the water supply by about 1/3rd at Carabooda, WA, using stomatal scheduling, reduced yield by about the same proportion whether trees were 'cross-' or 'self pollinated'.

Since stomata are so sensitive to water deficit, we decided to use stomatal conductance to schedule irrigation and compared it with 120% class A pan scheduling at Carabooda, WA. We chose 25% of full conductance as the level at which water should be applied, but this level is rather arbitrary. We found that over a season, this scheduling reduced the water applied by one third. In the 120% A pan treatment stomata remained open, except in the hot weather in February and March when stomatal conductance was similar in both treatments (Fig 4). Scheduling irrigation by using stomatal conductance reduced leaf gas exchange and reduced the number of fruit on the tree (Fig 3).

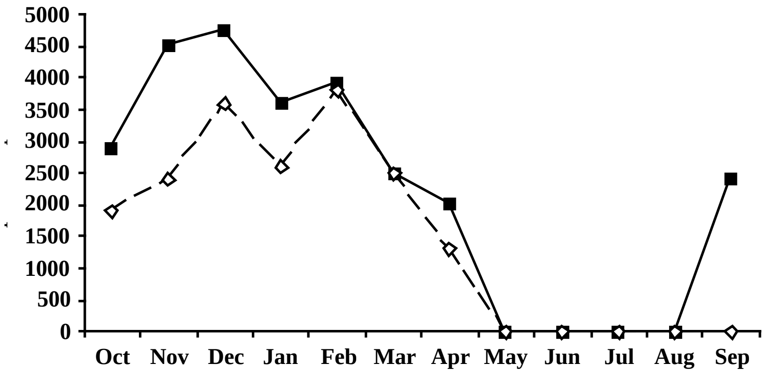

Fig. 4. The trees scheduled by the stomatal method (open symbols) received 1/3rd less water (L/tree) than those receiving 120% $A_{pan}$ (closed symbols).

*Partial root system drying*
In the glasshouse and field experiment, the soil on the dry side of the wet/dry treatments became as dry as that in the dry/dry treatment. In the glasshouse experiment, water use of the wet/dry treatment was about half that of the wet/wet. Despite this, the leaf water status, shoot and leaf growth, leaf gas exchange of these plants were the same as those in the wet/wet treatment. The plants exposed to the dry/dry treatment contained tyloses (gummy blockages) in the xylem vessels that conduct the water through the stems (please see photographs in the PowerPoint presentation of this paper on this CD-ROM). No tyloses were observed in the stems of the wet/wet or the wet/dry plants. Neither the wet nor the dry side of the wet/dry treatment had any tyloses. Thus, the wet/dry plants behaved the same as the wet/wet except that they used only half the amount of water.

The fluorescent dyes indicated that the tyloses in the xylem restricted water flow in the stems and this was confirmed by measurements of hydraulic conductivity (Fig 5). Indeed, the fluorescent dyes showed restrictions to water movement in the leaf petioles as well. These were due to embolisms (please see photographs in the PowerPoint presentation of this paper on this CD-ROM).

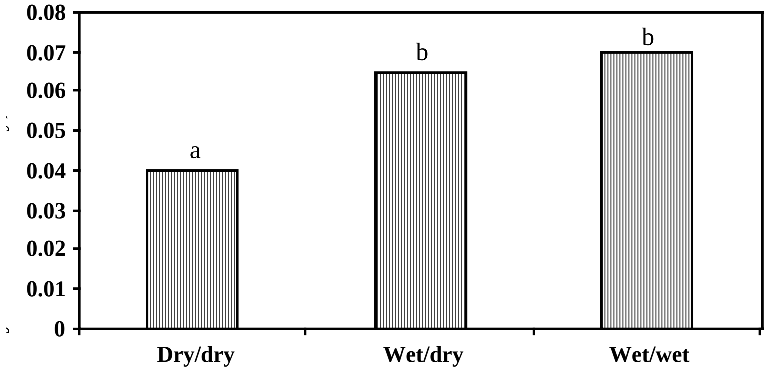

Fig. 5. Drying only one side of the root system did not significantly affect the ability of the stems to conduct water.

The plastic sheeting, used for the dry treatments, allowed the soil to dry to near wilting point. This was true for the dry section of the wet/dry treatments as well as the dry/dry treatments. We conclude that the plastic sheeting was effective and the wet/dry treatment was as expected. The physiological activity of the trees in the field was similar to those in the glasshouse. That is, the physiological activity of the wet/dry trees was the same as the wet/wet trees.

At the end of the season the wet/wet trees had more than 600 fruit/tree while many fruit dropped from the dry/dry (~200 fruit remaining per tree) and the wet/dry trees (~150 fruit remaining per tree). Thus, the wet/dry trees were similar to the wet/wet in almost all respects that we measured, except yield (Fig 6)

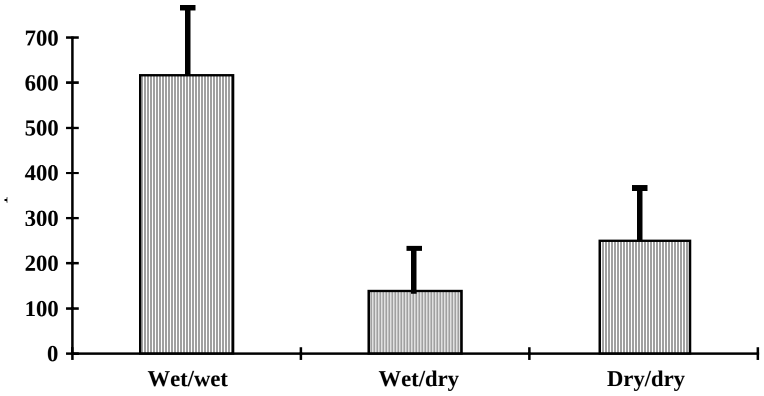

Fig. 6. Drying the root system for several months, either wholly or partially, caused fruit to drop from Hass trees at Carabooda, WA.

## What do we think it means?

*Reproductive and vegetative growth*

We expected reproductive growth to be very sensitive to water deficit but we found it to be surprisingly tough. During a slow drying cycle in the field in Qld, the young fruit continued to grow while vegetative growth was reduced and transpiration ceased. Thus, we conclude that under short-term water stress, reproductive growth is favoured over vegetative growth.

At Carabooda, WA, cross-pollination increased fruit retention irrespective of the level of water supply. This means that irrigation efficiency was increased in these experiments, not by manipulating water supply, but by increasing the number of fruit retained on the tree through the process of cross pollination.

*Leaf physiology and soil water deficits*

Stomatal conductance was a useful, but hardly practical, method of scheduling irrigation in our field experiment in WA. There are a number of reasons for this. One is the level of stomatal conductance chosen, 25% open proved to be too severe. The choice of a higher level, perhaps 50%, may have been more suitable. This needs to be tested. A practical limitation of the method is the expensive equipment needed to measure stomatal conductance. To deal with this we tried a number of cheaper, alternative techniques, but they either did not work on avocado or were difficult to calibrate with stomatal conductance in the field. Importantly, this experiment showed the close association between reduced leaf gas exchange and reduced yield. When water was reduced by 1/3rd, yield was reduced by the same proportion, showing the close link between stomatal physiology and productivity, in this environment.

*Partial root system drying*

By allowing only part of the root system to dry, we were able to maintain leaf physiological activity and hydraulic conductivity of the stems. This was true for plants in the glasshouse experiment and trees in the field experiment. The tyloses (gummy blockages of the xylem) were permanent because they did not disappear on re-watering. Thus, the dry/dry trees 'remembered' the impact of the drought. Thus, the wet/dry trees respond differently from those experiencing dry conditions over all the root system. We have the potential to translate this physiological activity into increased yield.

In a number of crops, partial root drying produces root signals, perhaps abscisic acid, that slow growth independently of the effects of the water deficit (Davies and Zhang 1991). Our data of leaf physiology and shoot and leaf growth of the wet/dry plants and trees are not consistent with the production of root signals. However, we have no explanation for the large fruit drop of the wet/dry trees which was similar to that of the dry/dry trees. It may be that 'signals' were generated over time and were sufficient to cause fruit to drop. Our measurements of these phenomema were separated in time and this may be significant in their interpretation. The measurements of growth and physiological activity were made in the first months after treatments were applied (summer-autumn), whereas yield was recorded eight months later (mid spring). For partial root drying to be a practical proposition in irrigation management the issue of fruit drop needs to be addressed.

## Towards the 2020 vision

The importance of this can be illustrated by comparing the yield response of our field experiments in WA, on a relative basis (Fig 7). This shows that when the whole root system experiences drought, yield and physiological activity are reduced proportionately. This close association will make it difficult to improve irrigation efficiency in a traditional irrigation management system.

On the other hand, the separation of yield and physiological activity in the partially dry root system treatment offers the possibility of improving irrigation efficiency. The way forward may be to reduce the length of time any one part of the root system is partially dry while retaining the benefits of reduced irrigation inputs.

### *Some uncertainties*

Pollination experiments

An important aspect of research is to be sure that the treatments are as reported. This is an issue for the cross and self pollinated trees in the field experiment at Carabooda where, a difference in yield was recorded. However, can we be certain that pollination was the cause of the difference? Unlike the trees in the glasshouse experiment at Nambour, the trees at Carabooda were not hand pollinated. Therefore, we cannot be certain that the trees with more fruit were indeed cross pollinated. DNA samples will be analysed to determine the pollination source.

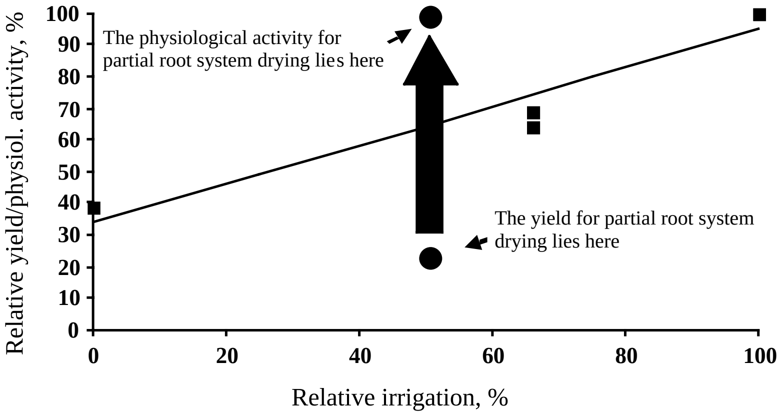

Fig 7. At Carabooda, WA, yield and physiological activity are reduced when water supply is reduced over the whole root system. For partially dry root systems, physiological activity and yield are separated. The challenge for 2020 is to match the yield with the physiological activity.

Scheduling according to stomatal conductance
Reduced water application for a season increased the chloride concentrations in leaves and fruit. There was an associated increase in marginal scorching of the leaves. Is the reduction in yield we observe due to the effect of the reduced water or due to the increased chloride in the tissues?

Partial root system drying
In the orchard at Carabooda, WA, nutrients are applied through the irrigation system. Hence in our experiments, reducing the water supply reduced the amount of nutrients the trees received. We measured leaf and fruit nutrient concentrations to see whether this effect could be measured. These data showed that in trees with partially dry root systems, while water flowed readily from one side of the tree to the other, this was not the case for nutrients. Therefore, fruit nutrition would need to be considered in any investigations of partial root system drying in avocado.

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